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(71) Applicants *(for all designated States except US)*: GENETIC THERAPY, INC. [US/US]; 938 Clopper Road, Gaithersburg, MD 20878 (US). UNIVERSITY OF CINCINNATI [US/US]; College of Medicine, Elland and Bethesda Avenues, Cincinnati, OH 45229-2899 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TRAPNELL, Bruce [US/US]; 4023 Byrd Road, Kensington, MD 20895 (US). WHITSETT, Jeffrey [US/US]; 5565 Salem Road, Cincinnati, OH 45230 (US).

(74) Agents: OLSTEIN, Elliot, M. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068-1739 (US).

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(57) Abstract

An adenoviral vector including a DNA sequence encoding a lung surfactant protein. The adenoviral vector may be a replication deficient adenoviral vector which is free of at least the majority of the E1 and E3 DNA sequences. Such vectors may be employed for generation of infectious viral particles which may transduce lung epithelial cells *in vivo* to enable the expression of lung surfactant protein by such cells.

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ADENOVIRAL VECTORS INCLUDING DNA ENCODING LUNG SURFACTANT PROTEIN

This application is a continuation-in-part of application Serial No. 08/044,406, filed April 8, 1993.

This invention relates to adenoviral vectors. More particularly, this invention relates to adenoviral vectors which include DNA encoding a lung surfactant protein and to the use of such vectors in treating disease states associated with lung surfactant protein deficiency, such as infant respiratory distress syndrome, and adult respiratory distress syndrome.

Surfactant proteins are natural endogenous proteins produced primarily within the alveolar and airway epithelial cells of the normal lung and interact with phospholipids to maintain the patency of the alveolar structures. When pulmonary surfactant protein concentration on the alveolar surface falls below critical levels, surface tension of the liquid-gas interface increases, thereby leading to alveolar collapse, pulmonary ventilation-perfusion mismatch, and hypoxia. In severe cases, this can lead to death. Intermittent administration of exogenous bovine lung surfactant protein has shown partial, but not

complete remission of the pathophysiology of the surfactant deficiency state.

It is an object of the present invention to provide a recombinant expression vehicle for expressing pulmonary surfactant protein.

It is a further object of the present invention to provide an expression vehicle which will enable prolonged expression of pulmonary surfactant protein in the lung in order to correct the clinical surfactant protein deficiency state and its attendant pathophysiologic effects on gas exchange.

The above objects and others should be apparent from the following specification.

In accordance with an aspect of the present invention, there is provided an adenoviral vector including a DNA sequence encoding a lung surfactant protein.

In one embodiment, the adenoviral vector is a replication deficient adenoviral vector, i.e., such vector is free of a DNA sequence(s) which is (are) required for viral replication, such as, for example, the E1 DNA sequence or a portion thereof. In one embodiment, the adenoviral vector is free of at least a portion of the adenoviral E1 DNA sequence and is free of at least a portion of the adenoviral E3 DNA sequence. The E3 region encodes several polypeptides which help the adenovirus to evade the immune surveillance of the host.

In one embodiment, the adenoviral vector comprises an adenoviral 5' inverted terminal repeat, or ITR; an adenoviral 3'

ITR; an adenoviral encapsidation signal; the DNA sequence encoding a lung surfactant protein; and a promoter controlling the expression of the DNA sequence encoding the lung surfactant protein. The vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and is not free of DNA sequences encoding adenoviral proteins expressed by the adenoviral major late promoter. In one embodiment, the vector is also free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences. In another embodiment, the vector is free of the adenoviral E1 and E3 DNA sequences, and is free of one of the E2 and E4 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

In yet another embodiment, the vector is free of at least the majority of the E1 and E3 DNA sequences, is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences, and is free of DNA sequences encoding adenoviral proteins expressed under control of the adenoviral major late promoter.

The DNA sequence encoding a lung surfactant protein is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus promoter; the respiratory

syncytial virus promoter; inducible promoters, such as the mouse mammary tumor virus, or MMTV, promoter; the metallothionein promoter; and heat shock promoters. In addition, tissue-specific promoters such as, but not limited to, lung surfactant protein promoters, may also be employed. It is to be understood, however, that the scope of the present invention is not to be limited to any specific promoter.

Lung surfactant proteins which may be encoded by the DNA sequence encoding a lung surfactant protein include surfactant protein A (SPA), surfactant protein B (SPB), and surfactant protein C (SPC).

Surfactant protein A is described in Kuroki, et al., J. Biol. Chem., Vol. 263, No. 7, pgs. 3388-3394 (March 5, 1988). Surfactant protein B and DNA encoding therefor are described in Pilot-Matias, et al., DNA, Vol. 8, No. 2, pgs. 75-86 (1989), Glasser, et al., Proc. Nat. Acad. Sci., Vol 84, pgs. 4007-4011 (June 1987); Revak, et al., J. Clin. Invest., Vol. 81, pgs. 826-833 (March 1988); O'Reilly, et al., Biochimica et Biophysica Acta, Vol. 1011, pgs. 140-148 (1989); and Weaver, et al., J. Amer. Phys. Soc., pgs. 982-987 (1988). Surfactant protein C is described further in Glasser, et al., J. Biol. Chem., Vol. 263, No. 21, pgs. 10326-10331 (July 25, 1988).

In one embodiment, the DNA sequence encoding a lung surfactant protein encodes lung surfactant protein B. Present evidence suggests that SPB is the most clinically important lung surfactant protein of those herein above described.

Such a vector, in a preferred embodiment, is assembled first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the leftward adenoviral genomic elements, the "critical left end elements", which include an adenoviral 5′ ITR, an adenoviral encapsidation signal, and the E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a tripartite leader sequence, a multiple cloning site; a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. Such DNA segment serves as a substrate for homologous recombination with a modified or mutated adenovirus, and such sequence may encompass, for example, a segment of the adenoviral genome from base 3328 to base 6241 of the adenovirus 5 genome. The plasmid may also include a selectable marker and an origin of replication. The origin of replication, may be, for example, a bacterial origin of replication. A representative example of such a shuttle plasmid is pAVS6, shown in Figure 4. An intron may be included within the transcribed portion to enhance the cytoplasmic mRNA accumulation levels.

The multiple cloning site facilitates the insertion of the DNA sequence encoding a lung surfactant protein into the plasmid. The DNA sequence encoding the lung surfactant protein may be inserted into the multiple cloning site. In general, restriction enzyme sites separating the above-mentioned components of the shuttle plasmid include "rare" restriction enzyme sites; i.e., sites which are found to occur randomly in

eukaryotic genes at a frequency from about one in every 10,000 to about one in every 100,000 base pairs. This increases the flexibility and ease of rearranging components of the vectors in assembled shuttle plasmids.

Homologous recombination is then effected with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted, as shown, for example, in Figure 8. Such homologous recombination may be effected through co-transfection of the shuttle plasmid and the modified adenovirus into a helper cell line, such as 293 (embryonic kidney epithelial) cells, by $CaPO_4$ precipitation. Upon such homologous recombination, a cloning vector is formed in which the modified adenovirus DNA which was 5' to the DNA segment in the shuttle plasmid corresponding to a similar segment of the modified adenoviral genome is replaced with the components in the shuttle plasmid which are 5' to such DNA segment. This homologous recombination, or "crossing over" event, can occur anywhere along the segment of the genome of the modified adenovirus which corresponds to the segment which is also contained within the shuttle plasmid (such as, for example, bases 3328 to 6241 of adenovirus 5 in Example 1 shown below).

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR; an adenoviral encapsidation signal; an Ela enhancer sequence; a promoter; a tripartite leader sequence; a DNA sequence encoding a lung surfactant protein; a poly A signal; adenoviral DNA free of at least the majority of

the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. This vector may then be introduced into a cell line such as the 293 cell line for production of large amounts of infectious recombinant adenoviral particles. The 293 cell line is a human fetal kidney epithelial cell line into which has been permanently introduced 11% of the left end of the adenovirus 5 genome. This directs the synthesis of the adenoviral E1a and E1b proteins and allows trans-complementation of E1-deleted vectors.

The infectious viral particles may then be administered to a host in vivo as part of a gene therapy procedure. Such infectious viral particles may be administered systemically, such as by intravenous or intraperitoneal or intrasmuscular or subcutaneous administration, or may be administered topically, such as by intratracheal or intrabronchial administration, or, alternatively, the infectious viral particles may be administered in an aerosol formulation. The infectious viral particles may be administered in an amount of up to about 10^{13} pfu, preferably from about 10^{7} pfu to about 10^{12} pfu. For example, the infectious viral particles may be employed in the transduction of the epithelium of the respiratory tract or alveoli; whereby the lung epithelial cells will express lung surfactant protein in amounts sufficient to achieve clinical correction of lung surfactant protein deficiency.

In addition, the infectious viral particles may be used to transduce eukaryotic cells in vitro. Eukaryotic cells which may be transduced include, but are not limited to, macrophages,

lymphocytes, fibroblasts, liver cells, bronchial cells, and other epithelial or endothelial cells. Such eukaryotic cells then may be administered to a host as part of a gene therapy procedure, or may be cultured in vitro whereby such cells produce lung surfactant protein.

In addition, the infectious viral particles may be used to transduce eukaryotic cells in vitro for the in vitro production of lung surfactant protein. Examples of eukaryotic cells which may be transduced in vitro for the in vitro production of lung surfactant protein include, but are not limited to, those eukaryotic cells hereinabove described, as well as Chinese Hamster Ovary (CHO) cells, COS-7 cells, NIH 3T3 cells, vero cells, HeLa cells, MRC-5 cells, CN1 cells, W138 cells, and chicken lymphoma cells. The lung surfactant protein produced by such cells may then be administered to a host in conjunction with an acceptable pharmaceutical carrier in order to treat lung surfactant protein deficiency states.

In another embodiment, the vector comprises an adenoviral 5′ ITR; an adenoviral 3′ ITR; an adenoviral encapsidation signal; a DNA sequence encoding a lung surfactant protein; and a promoter controlling the DNA sequence encoding a lung surfactant protein. The vector is free of the adenoviral E1, E2, E3, and E4 DNA sequences, and the vector is free of DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter; i.e., the vector is free of DNA encoding adenoviral

structual proteins. Such vector is sometimes hereinafter referred to as a "gutless adenoviral vector," or "GLAd" vector.

Promoters which are contained in the vector may be those hereinabove described.

Such vectors may be constructed by removing the adenoviral 5′ ITR, the adenoviral 3′ ITR, and the adenoviral encapsidation signal, from an adenoviral genome by standard techniques. Such components, as well as a promoter (which may be an adenoviral promoter or a non-adenoviral promoter), tripartite leader sequence, poly A signal, may, by standard techniques, be ligated into a base plasmid or "starter" plasmid such as, for example, pKSII⁻(Strategene), to form an appropriate cloning vector. The cloning vector may include a multiple cloning site, as hereinabove described, to facilitate the insertion of the foreign DNA sequence into the cloning vector. An appropriate vector in accordance with the present invention is thus formed by cutting the cloning vector by standard techniques at appropriate restriction sites in the multiple cloning site, and then ligating the DNA sequence encoding a lung surfactant protein into the cloning vector.

The GLAd vector may then be packaged into infectious viral particles using a helper adenovirus or cell line which provides the necessary packaging materials. If a helper virus is used, in one embodiment, preferably it has a defective encapsidation signal in order that the helper virus will not package itself. Examples of such encapsidation-defective helper viruses which may

be employed are described in Grable, et al., J. Virol., Vol. 66, pgs. 723-731 (1992), and in Grable, et al., J. Virol., Vol. 64, pgs. 2047-2056 (1990). In another embodiment, the helper virus has a normal packaging signal.

DNA for the vector and the encapsidation-defective helper virus are transfected into an appropriate cell line for the generation of infectious viral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes. Examples of appropriate cell lines include, but are not limited to, HeLa cells, A549 cells, or 293 (embryonic kidney epithelial) cells. The infectious viral particles may then be purified away from helper virus by CsCl isopycnic density centrifugation and transduced into lung epithelial cells lining the respiratory tract or alveoli, as hereinabove described, whereby such cells express lung surfactant protein.

In another alternative, the vector is transfected into the cells, followed by infection of the cells with the encapsidation-defective helper virus.

The invention will now be described with respect to the following example, however, the scope of the present invention is not intended to be limited thereby.

Example 1

The adenoviral construction shuttle plasmid pAvS6 was constructed in several steps using standard cloning techniques including polymerase chain reaction based cloning techniques.

First, the 2913 bp BglII, HindIII fragment was removed from Ad-d1327 and inserted as a blunt fragment into the XhoI site of pKSII˙ (Stratagene, La Jolla, CA) (Figure 1). Ad-d1327 (Thimmappaya, et al., Cell, Vol. 31, pg. 543 (1983)) is identical to adenovirus 5 except that an XbaI fragment including bases 28593 to 30470 (or map units 78.5 to 84.7) of the adenovirus 5 genome, and which is located in the E3 region, has been deleted.

The comlete Adenovirus 5 genome is registered as Genbank accession #M73260, incorporated herein by reference, and the virus is available from the American Type Culture Collection, Rockville, Maryland, U.S.A. under accession number VR-5.

Ad-d1327 was constructed by routine methods from Adenovirus 5 (Ad5). The method is outlined briefly as follows and previously described by Jones and Shenk, Cell 13:181-188 (1978). Ad5 DNA is isolated by proteolytic digestion of the virion and partially cleaved with Xba I restriction endonuclease. The Xba I fragments are then reassembled by ligation as a mixture of fragments. This results in some ligated genomes with a sequence similar to Ad5, except excluding sequences 28593 bp to 30470 bp. This DNA is then transfected into suitable cells (e.g. KB cells, HeLa cells, 293 cells) and overlaid with soft agar to allow plaque formation. Individual plaques are then isolated, amplified, and screened for the absence of the 1878 bp E3 region Xba 1 fragment. The orientation of this fragment was such that the BglII site was nearest the T7 RNA polymerase site of pKSII˙

and the HindIII site was nearest the T3 RNA polymerase site of pKSII⁻. This plasmid was designated pHR. (Figure 1).

Second, the ITR, encapsidation signal, Rous Sarcoma Virus promoter, the adenoviral tripartite leader (TPL) sequence and linking sequences were assembled as a block using PCR amplification (Figure 2). The ITR and encapsidation signal (sequences 1-392 of Ad-dl327 [identical to sequences from Ad5, Genbank accession #M73260]) were amplified (amplification 1) together from Ad-dl327 using primers containing NotI or AscI restriction sites. The Rous Sarcoma Virus LTR promoter was amplified (amplification 2) from the plasmid pRC/RSV (sequences 209 to 605; Invitrogen, San Diego, CA) using primers containing an AscI site and an SfiI site. DNA products from amplifications 1 and 2 were joined using the "overlap" PCR method (amplification 3) with only the NotI primer and the SfiI primer. Complementarity between the AscI containing end of each initial DNA amplification product from reactions 1 and 2 allowed joining of these two pieces during amplification. Next the TPL was amplified (amplification 4) (sequences 6049 to 9730 of Ad-dl327 [identical to similar sequences from Ad5, Genbank accession #M73260]) from cDNA made from mRNA isolated from 293 cells infected for 16 hours with Ad-dl327 using primers containing SfiI and XbaI sites respectively. DNA fragments from amplification reactions 3 and 4 were then joined using PCR (amplification 5) with the NotI- and XbaI-site-containing primers, thus creating the complete gene block.

Third, the ITR-encapsidation signal-TPL fragment was then purified, cleaved with NotI and XbaI and inserted into the NotI, XbaI cleaved pHR plasmid. This plasmid was designated pAvS6A and the orientation was such that the NotI site of the fragment was next to the T7 RNA polymerase site (Figure 3).

Fourth, the SV40 early polyA signal was removed from SV40 DNA as an HpaI-BamHI fragment, treated with T4 DNA polymerase and inserted into the SalI site of the plasmid pAvS6A-(Figure 3) to create pAvS6 (Figures 3 and 4).

A 2kb DNA fragment containing the whole human pulmonary surfactant protein B (SPB) cDNA (Figure 5) (Pilot-Matias, 1989) was obtained from plasmid pKC4-SPB (Figure 6) (Weaver, et al., J. Amer. Phys. Soc., pgs. L-95 to L-103 (1992)) by EcoRI digestion. This DNA fragment was isolated, purified, and then cloned into the EcoRV site of plasmid pAVS6. (Figure 4). Three identical clones with correct insertion of SPB cDNA were obtained. Such clones are named pAVS6·SPB#7, pAVS6·SPB#12, and pAVS6·SPB#13. pAVS6·SPB#7 is shown in Figure 7. The orientation of the SPB DNA within the shuttle plasmid was obtained by evaluating the DNA sequences of the two termini of the SPB cDNA insert in the plasmid with primers derived from pAVS6.

The recombinant adenoviral vector AV1SPB1 (Figure 8), containing SPB cDNA was constructed through homologous recombination between the Ad5 deletion mutant Ad-dl327 (Figure 8), and pAVS6·SPB#7. Homologous recombination, or "crossing over," occurs between Ad-dl327 and pAVS6.SPB#7, along the

segment common to both Ad-dl327 and pAVS6.SPB#7 which corresponds to bases 3328 to 6241 (or map units 9.24 to 17.34) of the adenovirus 5 genome. Ad-dl327 has a deleted E3 region in which base pairs 28593 to 30470 are absent (Thimmappaya, et al, Cell, Vol. 31, pgs. 543-551 (1982)). pAVS6·SPB#7 contains an adenoviral 5' ITR, an origin of replication contained completely within the 5' ITR, an E1a enhancer and encapsidation signal, a Rous Sarcoma Virus promoter, an adenovirus 5 tripartite leader sequence and the 2kb human SPB cDNA including the entire protein coding sequence (nucleotides 1 to 1172), and the SV40 poly A signal.

Example 2

293 cells (ATCC No. CRL 1573) were infected with AV1SPB1 at a multiplicity of infection (MOI) of 50 MOI units. At 12 hours post-infection, the cells were radiolabeled with ^{35}S-methionine (50μCi/ml) overnight. Identical amounts of labeled protein were used for immunoprecipitation with antisera against SPB. Immunopreciptates were analyzed by SDS-polyacrylamide gel electrophoresis on 16% gel. The gels were fluorographed. C^{14}-labeled molecular weight markers and BioRad broad range molecular weight markers were used as size markers. As shown in Figure 9, lane 1 shows an uninfected control; lane 2 shows AV1SPB1-infected 293 cells in which electrophoresis of immunoprecipitates occurred under reducing conditions; and lane 3 shows AV1SPB1-infected 293 cells in which electrophoresis of immunoprecipitates occurred under non-reducing conditions. The active SPB peptide migrates at approximately Mr=6,000 to 8,000 (reduced) and forms oligomers

(unreduced) identical to that of native human SPB (Mr=18,000). The precursor protein also was detected in both reduced and unreduced conditions.

Example 3

Mouse lung Type II-like epithelial cell lines were transduced with adenoviral vector AV1SPB1, which contains the full length human SPBcDNA under the control of the Rous Sarcoma Virus promoter. Expression of SPB was assessed by RNase protection using ^{32}P-labelled probes specific for endogenous mouse SPB (upper band) or human SPB (middle band). A B-tubulin specific probe (lowest band) also was used to ensure that the same amount of RNA was added to each assay. The B-tubulin probe did not recognize human B-tubulin in lane 1. As shown in Figure 10, cells infected with AV1SPB1 at 50 multiplicity of infection (MOI) units (lane 2), 100 MOI units (lane 3), and 150 MOI units (lane 4), clearly expressed human SPB mRNA. Human SPB was not detected in uninfected cells (lane 5).

Example 4

Four cotton rats were anesthetized by metaphane, and were given 1.5 x 10^{10} pfu of Av1SPB1. The rats were sacrificed at 2 (n=1), 3 (n=2) or 45 (n=1) days after administration, and the lungs were harvested. Total lung RNA then was extracted using the guanidine thiocyanate-CsCl technique (Chirgwin, et al., Biochemistry, Vol. 18, pgs. 5294-5299 (1979)). Total lung RNA(15 μg) was subjected to formaldehyde agarose gel electrophoresis and transferred to a nylon membrane (Nytran,

Schleicher and Schuell). The filter was crosslinked (UV Crosslink, Stratagene), and hybridized with a ^{32}P-labeled 2.0 kb human SP-B cDNA probe prepared by random priming (Loftstrand) and evaluated by autoradiography. Lungs from uninfected control rats and from rats infected with Av1LacZ4 also were subjected to the above hybridization procedure.

Northern blot analysis, as shown in Figure 11, demonstrated that human SP-B mRNA was expressed in the lungs of cotton rats infected with Av1SP-B1, but not in those of uninfected animals nor in animals infected with Av1LacZ4. A 2.0 kb mRNA for the human SP-B gene was detected in cotton rats infected with Av1SP-B1, consistent with the size of the full length human SP-B mRNA transcribed by the vector.

Example 5

Two cotton rats were anesthetized with metaphane, and Av1SP-B1 (diluted in PBS to 10^{10} pfu/300μl) was administered via intranasal instillation. Two days after infection, the rats were sacrificed and the lungs were harvested. The lungs were washed twice in PBS and perfused with methionine-free LHC-8 medium (Biofluids), minced, and incubated for 24 hours in medium with ^{35}S-Cys/Met (1ml of medium, 100μCi/ml). The presence of SP-B in media and lysed lung explants was assessed by immunoprecipitation with rabbit anti-human SP-B antiserum to detect the secreted SP-B and processed SP-B peptides. (Weaver, et al., Am. J. Physiol., Vol. 263, pgs. L95-L103 (1992)). Av1LacZ4 treated rats and untreated rats were used as controls.

As shown in Figure 12, de novo synthesis and secretion of human SP-B peptide was detected from lung fragments removed from animals infected with Av1SPB1, and was not detected in uninfected animals or in animals infected with Av1LacZ4. Human SP-B peptide was detected as secreted, with 8 kda and 18 kda oligomeric forms suggesting that vector derived precursor SP-B (proSP-B) was processed after in vivo adenoviral vector-mediated gene transfer.

Example 6

Cotton rats were treated intranasally with Av1SP-B1 in amounts of (a) 0.5 x 10^9 pfu (n=1); (b) 1.5 x 10^9 pfu (n=1); or (c) 1.5 x 10^{10} pfu (n=1). The animals were sacrificed 48 hours after infection, and the lungs were prepared for in situ hybridization analysis according to the method of Wert, et al., Development Biology, Vol. 156, pgs. 426-443 (1993), using human SP-BcRNA. An uninfected rat was used as a control.

Lung sections were inflation fixed in 20 cm of water in 4% paraformaldehyde at 4°C overnight. Radiolabeled ribroprobe for human SP-B was generated by in vitro transcription in the presence of ^{35}S-VTP. Slides were hybridized to the human SP-B probe under stringent conditions and exposed to emulsion from one to eight days, after which the slides were counterstained with hematoxylin and eosin. Antisense and sense probes were compared in Av1SPB1-treated, in Av1LacZ4-treated, and in control (untreated) animals.

In situ hybridization of the Av1SP-B1 infected cotton rats demonstrated expression of human SP-B mRNA in bronchiolar and

alveolar epithelial cells. Human SP-B mRNA was detected in a patchy distribution and increased in a dose-dependent manner, as shown in Figures 13 through 16, which show the in situ hybridization results of the rats treated with 0.5 x 10^9 pfu of Av1SP-B1; 1.5 x 10^9 pfu of Av1SP-B1; 1.5 x 10^{10} pfu of Av1SP-B1; and an uninfected control rat, respectively. Light microscopic analysis of the lungs of Av1SP-B1 or Av1LacZ treated animals demonstrated a mild inflammatory response with a peribronchiolar lymphomonocytic infiltrate, increased macrophages, and some polymorpholeukocytes. The infiltrates were prominent after 48 hours and were dose-dependent. In parallel experiments with Av1lacZ4, the infiltrates were essentially resolved 3 to 4 weeks after exposure.

All patents, publications, and database entries referenced in this specification are indicative of the level of skill of persons in the art to which the invention pertains. The disclosures of all such patents, publications (including published patent applications), and database entries are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced

other than as particularly described and still be within the scope of the accompanying claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Trapnell, Bruce
Whitsett, Jeffrey

(ii) TITLE OF INVENTION: Adenoviral Vectors Including DNA Encoding Lung Surfactant Protein

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart, & Olstein

(B) STREET: 6 Becker Farm Road

(C) CITY: Roseland

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07068

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Olstein, Elliot M.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2,016 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(ix) FEATURE:

(A) NAME/KEY: Surfactant protein B cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACGGGTCCCG GGACTCAAGA CCACGGTTTC GGACCTCGTT CGTAACGTCA CGTCTCGGGA

AGGGCATTGC CTACAGGAAG TCTGGGGACA TGTGGGAGCC GATGACCTAT GCCAAGAGTG    240
TCCCGTAACG GATGTCCTTC AGACCCCTGT ACACCCTCGG CTACTGGATA CGGTTCTCAC

TGAGGACATC GTCCACATCC TTAACAAGAT GGCCAAGGAG GCCATTTTCC AGGACACGAT    300
ACTCCTGTAG CAGGTGTAGG AATTGTTCTA CCGGTTCCTC CGGTAAAAGG TCCTGTGCTA

GAGGAAGTTC CTGGAGCAGG AGTGCAACGT CCTCCCCTTG AAGCTGCTCA TGCCCCAGTG    360
CTCCTTCAAG GACCTCGTCC TCACGTTGCA GGAGGGGAAC TTCGACGAGT ACGGGGTCAC

CAACCAAGTG CTTGACGACT ACTTCCCCCT GGTCATGGAC TACTTCCAGA ACCAGACTGA    420
GTTGGTTCAC GAACTGCTGA TGAAGGGGGA CCAGTAGCTG ATGAAGGTCT TGGTCTGACT

CTCAAACGGC ATCTGTATGC ACCTGGGCCT GTGCAAATCC CGGCAGCCAG AGCCAGAGCA    480
GAGTTTGCCG TAGACATACG TGGACCCGGA CACGTTTAGG GCCGTCGGTC TCGGTCTCGT

GGAGCCAGGG ATGTCAGACC CCCTGCCCAA ACCTCTGCGG GACCCTCTGC CAGACCCTCT    540
CCTCGGTCCC TACAGTCTGG GGGACGGGTT TGGAGACGCC CTGGGAGACG GTCTGGGAGA

GCTGGACAAG CTCGTCCTCC CTGTGCTGCC CGGGGCCCTC CAGGCGAGGC CTGGGCCTCA    600
CGACCTGTTC GAGCAGGAGG GACACGACGG GCCCCGGGAG GTCCGCTCCG GACCCGGAGT

CACACAGGAT CTCTCCGAGC AGCAATTCCC CATTCCTCTC CCCTATTGCT GGCTCTGCAG    660
GTGTGTCCTA GAGAGGCTCG TCGTTAAGGG GTAAGGAGAG GGGATAACGA CCGAGACGTC

GGCTCTGATC AAGCGGATCC AAGCCATGAT TCCCAAGGGT GCGCTACGTG TGGCAGTGGC    720
CCGAGACTAG TTCGCCTAGG TTCGGTACTA AGGGTTCCCA CGCGATGCAC ACCGTCACCG

CCAGGTGTGC CGCGTGGTAC CTCTGGTGGC GGGCGGCATC TGCCAGTGCC TGGCTGAGCG    780
GGTCCACACG GCGCACCATG GAGACCACCG CCCGCCGTAG ACGGTCACGG ACCGACTCGC

CTACTCCGTC ATCCTGCTCG ACACGCTGCT GGGCCGCATG CTGCCCCAGC TGGTCTGCCG    840
GATGAGGCAG TAGGACGAGC TGTGCGACGA CCCGGCGTAC GACGGGGTCG ACCAGACGGC

CCTCGTCCTC CGGTGCTCCA TGGATGACAG CGCTGGCCCA AGGTCGCCGA CAGGAGAATG    900
GGAGCAGGAG GCCACGAGGT ACCTACTGTC GCGACCGGGT TCCAGCGGCT GTCCTCTTAC

GCTGCCGCGA GACTCTGAGT GCCACCTCTG CATGTCCGTG ACCACCCAGG CCGGGAACAG    960
CGACGGCGCT CTGAGACTCA CGGTGGAGAC GTACAGGCAC TGGTGGGTCC GGCCCTTGTC

CAGCGAGCAG GCCATACTAC AGGCAATGCT CCAGGCCTGT GTTGGCTCCT GGCTGGACAG   1020
GTCGCTCGTC CGGTATGATG TCCGTTACGA GGTCCGGACA CAACCGAGGA CCGACCTGTC
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GGAAAAGTGC AAGCAATTTG TGGAGCAGCA CACGCCCCAG CTGCTGACCC TGGTGCCCAG 1080
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GGGCTGGGAT GCCCACACCA CCTGCCAGGC CCTCGGGGTG TGTGGGACCA TGTCCAGCCC 1140
CCCGACCCTA CGGGTGTGGT GGACGGTCCG GGAGCCCCAC ACACCCTGGT ACAGGTCGGG

TCTCCAGTGT ATCCACAGCC CCGACCTTTG ATGAGAACTC AGCTGTCCAG GTGCAAAGGA 1200
AGAGGTCACA TAGGTGTCGG GGCTGGAAAC TACTCTTGAG TCGACAGGTC CACGTTTCCT

AAAGCCAAGT GAGAGGGGCT CTGGGACCAT GGTGACCAGG CTCTTCCCCT GCTCCCTGGC 1260
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CCTCGCCAGC TGCCAGGCTG AAAAGAAGCC TCAGCTCCCA CACCGCCCTC CTCACCGCCC 1320
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TTCCTCGGCA GTCACTTCCA CTGGTGGACC ACGGGCCCCC AGCCCTGTGT CGGCCTTGTC 1380
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TGTCTCAGCT CAACCACAGT CTGACACCAG AGCCCACTTC CATCCTCTCT GGTGTGAGGC 1440
ACAGAGTCGA GTTGGTGTCA GACTGTGGTC TCGGGTGAAG GTAGGAGAGA CCACACTCCG

ACAGCGAGGG CAGCATCTGG AGGAGCTCTG CAGCCTCCAC ACCTACCACG ACCTCCCAGG 1500
TGTCGCTCCC GTCGTAGACC TCCTCGAGAC GTCGGAGGTG TGGATGGTGC TGGAGGGTCC

GCTGGGCTCA GGAAAAACCA GCCACTGCTT TACAGGACAG GGGGTTGAAG CTGAGCCCCG 1560
CGACCCGAGT CCTTTTTGGT CGGTGACGAA ATGTCCTGTC CCCCAACTTC GACTCGGGGC

CCTCACACCC ACCCCCATGC ACTCAAAGAT TGGATTTTAC AGCTACTTGC AATTCAAAAT 1620
GGAGTGTGGG TGGGGGTACG TGAGTTTCTA ACCTAAAATG TCGATGAACG TTAAGTTTTA

TCAGAAGAAT AAAAAATGGG AACATACAGA ACTCTAAAAG ATAGACATCA GAAATTGTTA 1680
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AGTTAAGCTT TTTCAAAAAA TCAGCAATTC CCAGCGTAGT CAAGGGTGGA CATGCACGCG 1740
TCAATTCGAA AAAGTTTTTT AGTCGTTAAG GGTCGCATCA GTTCCCACCT GTACGTGCGC

TCTGGCATGA TGGGATGGCG ACCGGGCAAG CTTTCTTCCT CGAGATCGTC TGCTGCTTGA 1800
AGACCGTACT ACCCTACCGC TGGCCCGTTC GAAAGAAGGA GCTCTAGCAG ACGACGAACT

GAGCTATTGC TTTGTTAAGA TATAAAAAGG GGTTTCTTTT TGTCTTTCTG TAAGGTGGAC 1860
CTCGATAACG AAACAATTCT ATATTTTTCC CCAAAGAAAA ACAGAAAGAC ATTCCACCTG

TTCCAGCTTT TGATTGAAAG TCCTAGGGTG ATTCTATTTC TGCTGTGATT TATCTGCTGA 1920
AAGGTCGAAA ACTAACTTTC AGGATCCCAC TAAGATAAAG ACGACACTAA ATAGACGACT

AAGCTCAGCT GGGGTTGTGC AAGCTAGGGA CCCATTCCTA TGTAATACAA TGTCTGCACC 1980
TTCGAGTCGA CCCCAACACG TTCGATCCCT GGGTAAGGAT ACATTATGTT ACAGACGTGG

AATGCTAATA AAGTCCTATT CTCTTTTATC GGAATT 2016
TTACGATTAT TTCAGGATAA GAGAAAATAG CCTTAA

WHAT IS CLAIMED IS:

1. An adenoviral vector including a DNA sequence encoding a lung surfactant protein.

2. The vector of Claim 1 wherein said lung surfactant protein is Surfactant Protein B.

3. The vector of Claim 1 wherein said adenoviral vector is a replication deficient adenoviral vector.

4. The vector of Claim 3 wherein said adenoviral vector is free of at least a portion of the adenoviral E1 DNA sequence, and is free of at least a portion of the adenoviral E3 DNA sequence.

5. The vector of Claim 4 wherein said vector comprises:

an adenoviral 5′ ITR;

an adenoviral 3′ ITR;

an adenoviral encapsidation signal;

a DNA sequence encoding a lung surfactant protein;

a promoter controlling said foreign DNA sequence, said vector being free of at least the majority of the adenoviral E1 and E3 DNA sequences, but not free of all of the E2 and E4 DNA sequences and DNA sequences encoding adenoviral proteins controlled by the adenoviral major late promoter.

6. The vector of Claim 5 wherein the vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences.

7. The vector of Claim 6 wherein said vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of one of the E2 and E4 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

8. The vector of Claim 7 wherein said vector is free of at least the majority of the E1 and E3 DNA sequences, is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences, and is free of DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter.

9. The vector of Claim 5 wherein said promoter is an adenoviral promoter.

10. The vector of Claim 5 wherein said promoter is a heterologous promoter.

11. Infectious viral particles generated from the vector of Claim 1.

12. Lung epithelial cells transfected with the infectious viral particles of Claim 11.

13. Infectious viral particles generated from the vector of Claim 5.

14. Lung epithelial cells transfected with the infectious viral particles of Claim 13.

15. A process for treating lung surfactant protein deficiency states, comprising:

administering to a host an effective amount of the infectious viral particles of Claim 11.

16. A process for treating lung surfactant protein deficiency states, comprising:

administering to a host an effective amount of the infectious viral particles of Claim 13.

17. The process of Claim 15 wherein said infectious viral particles are administered in an amount up to about 10^{13}pfu.

18. The process of Claim 17 wherein said infectious viral particles are administered in an amount of from about 10^{7}pfu to about 10^{12}pfu.

19. The process of Claim 16 wherein said infectious viral particles are administered in an amount of from 1 pfu to about 10^{12}pfu.

20. The process of Claim 19 wherein said infectious viral particles are administered in an amount of from about 10^{7}pfu to about 10^{12}pfu.

21. The process of Claim 15 wherein said lung surfactant protein deficiency state is infant respiratory distress syndrome.

22 The process of Claim 16 wherein said lung surfactant protein deficiency state is infant respiratory distress syndrome.

23. The process of Claim 15 wherein said lung surfactant protein deficiency state is adult respiratory distress syndrome.

24. The process of Claim 16 wherein said lung surfactant protein deficiency state is adult respiratory distress syndrome.

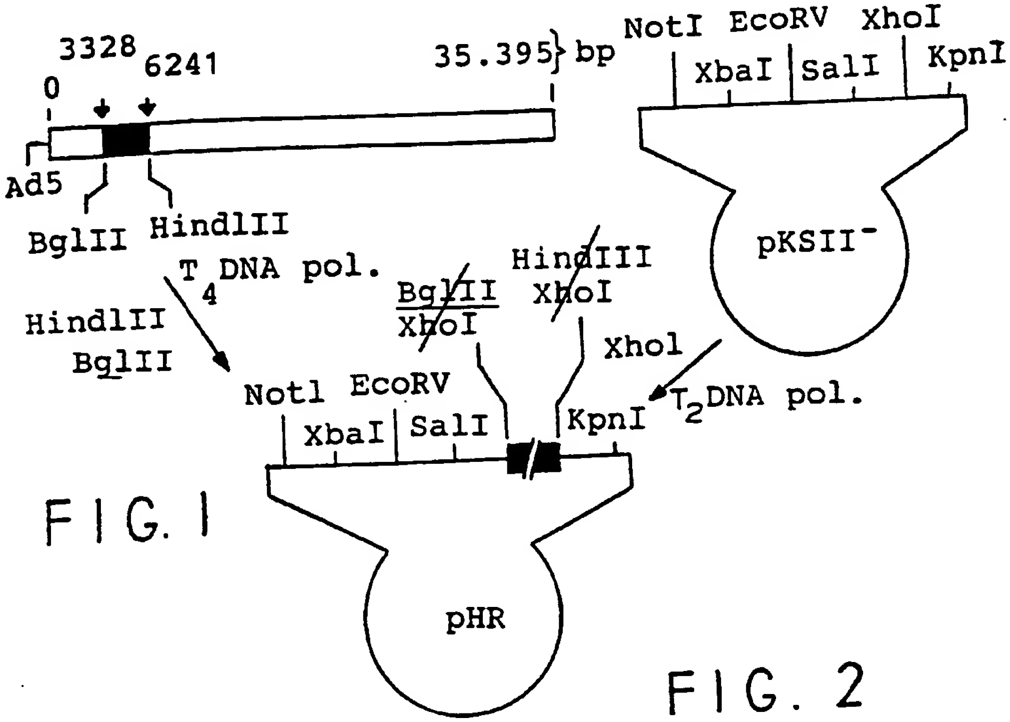

3328
6241
0
35.395} bp
Ad5
BglII
HindlII
NotI EcoRV XhoI
XbaI SalI KpnI
pKSII⁻
T4 DNA pol.
HindlII
BglII
BglII
XhoI
HindIII
XhoI
Xhol
T2 DNA pol.
Notl EcoRV
XbaI SalI
KpnI
pHR

FIG. 1

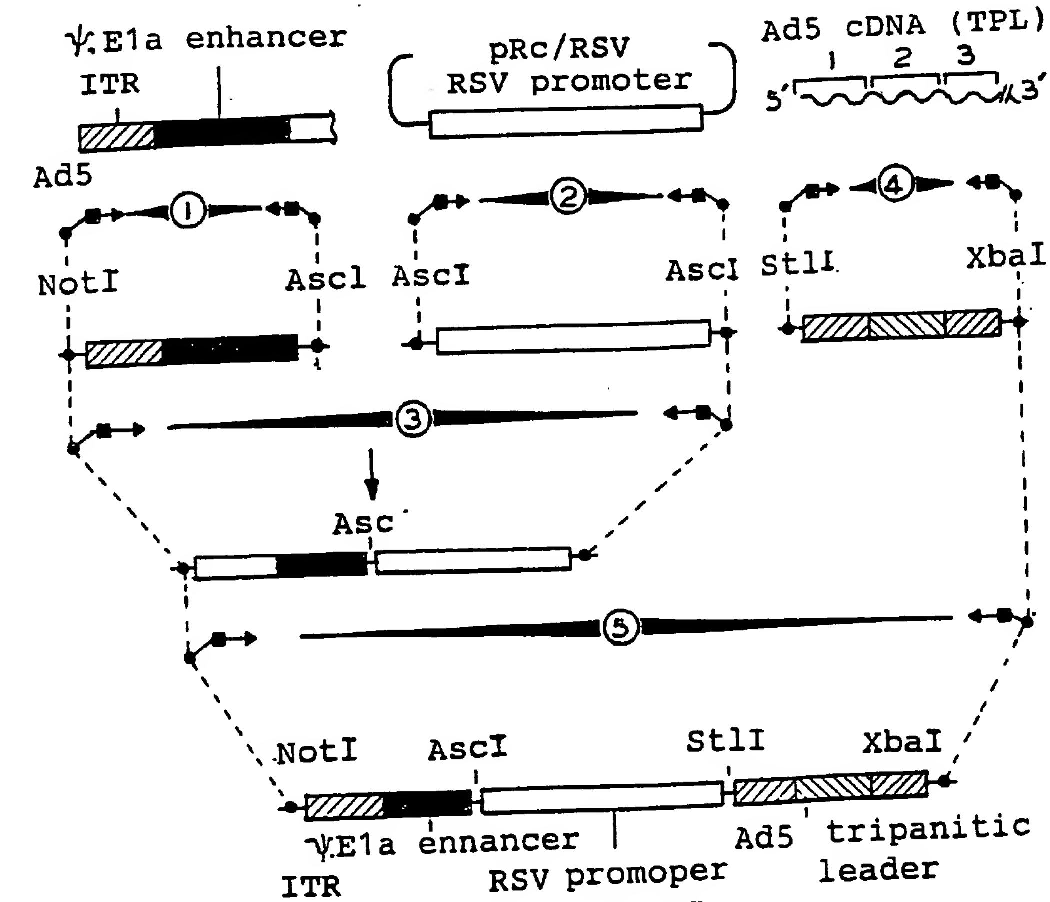

ψ E1a enhancer
ITR
Ad5
pRc/RSV
RSV promoter
Ad5 cDNA (TPL)
1 2 3
5' 3'
1
2
4
NotI
Ascl
AscI
AscI
StlI
XbaI
3
Asc
5
NotI
AscI
StlI
XbaI
ψE1a ennancer
ITR
RSV promoper
Ad5
tripanitic
leader

FIG. 2

FIG. 3

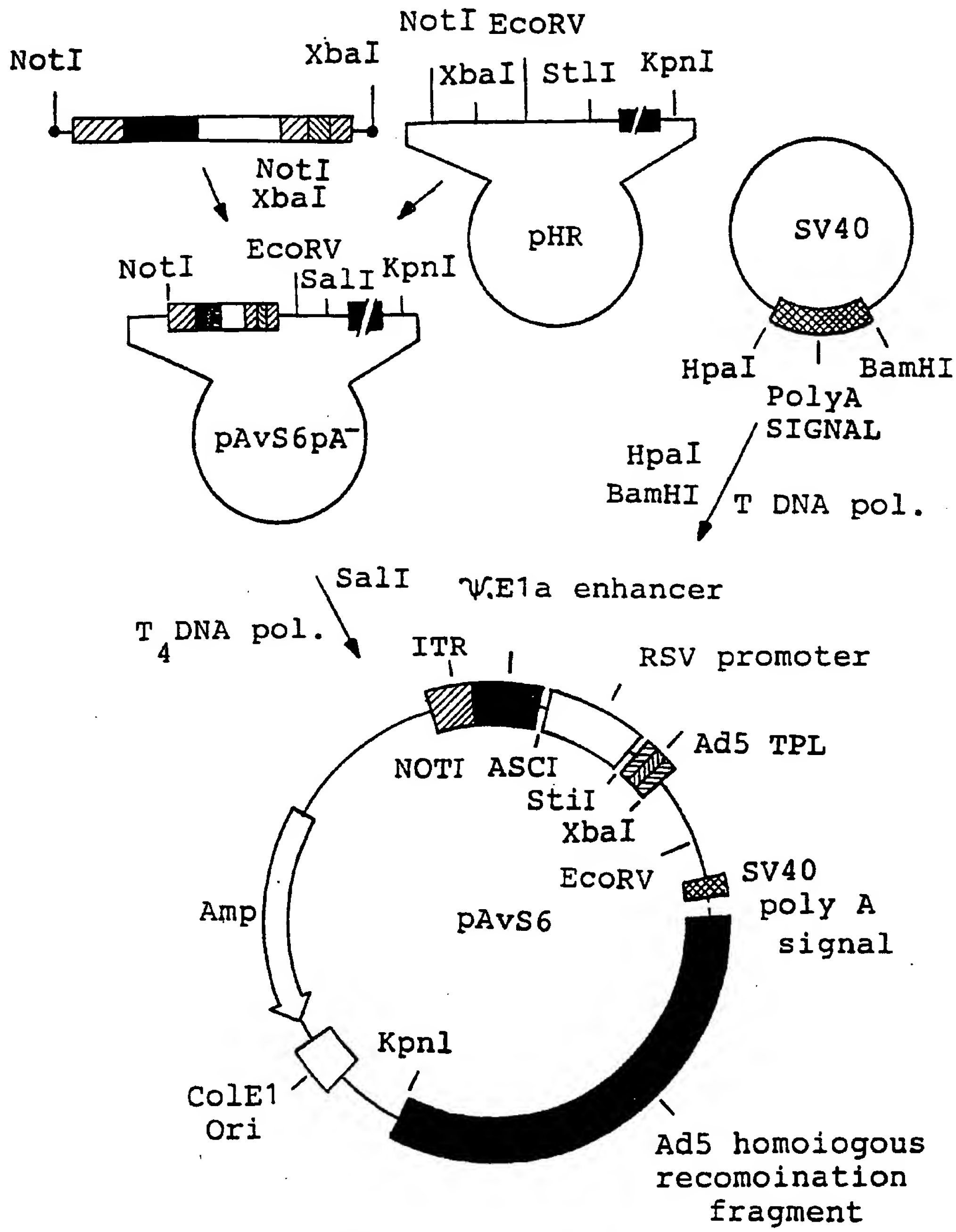

NotI
XbaI
NotI EcoRV
XbaI
StlI
KpnI
NotI
XbaI
pHR
SV40
NotI
EcoRV
SalI
KpnI
pAvS6pA⁻
HpaI
BamHI
PolyA
SIGNAL
HpaI
BamHI
T DNA pol.
SalI
ΨE1a enhancer
T4 DNA pol.
ITR
RSV promoter
NOTI
ASCI
Ad5 TPL
StiI
XbaI
EcoRV
SV40
poly A
signal
Amp
pAvS6
Kpnl
ColE1
Ori
Ad5 homoiogous
recomoination
fragment

FIG. 4

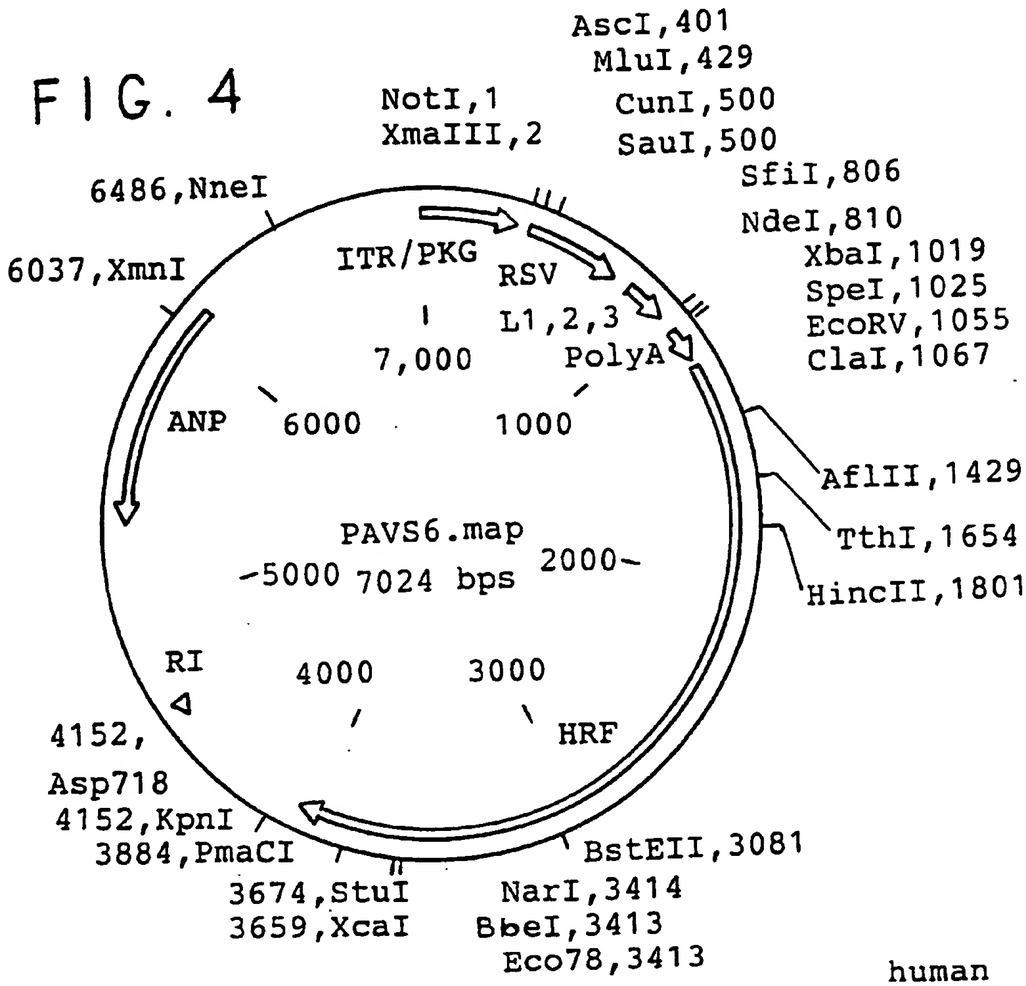
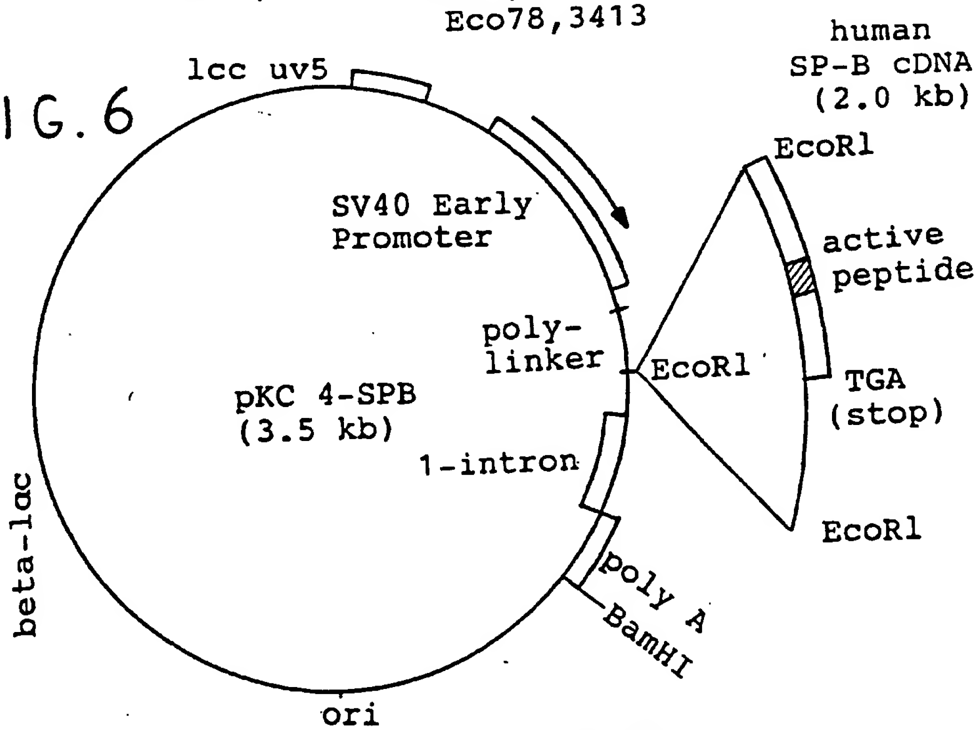

AscI,401
MluI,429
CunI,500
SauI,500
NotI,1
XmaIII,2
SfiI,806
NdeI,810
6486,NneI
XbaI,1019
SpeI,1025
EcoRV,1055
ClaI,1067
6037,XmnI
ITR/PKG
RSV
L1,2,3
PolyA
7,000
ANP
6000
1000
AflII,1429
PAVS6.map
7024 bps
TthI,1654
5000
2000
HincII,1801
RI
4000
3000
HRF
4152,
Asp718
4152,KpnI
3884,PmaCI
BstEII,3081
3674,StuI
3659,XcaI
NarI,3414
BbeI,3413
Eco78,3413

FIG. 6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(2.0 kb)
lcc uv5
EcoR1
SV40 Early
Promoter
active
peptide
poly-
linker
EcoR1
TGA
(stop)
pKC 4-SPB
(3.5 kb)
1-intron
EcoR1
beta-lac
poly A
BamHI
ori

*** SEQUENCE ***

FIG. 5A

```
  1  AATTCCGTCA AGCTGCAGAG GTGCCATGGC TGAGTCACAC CTGCTGCAGT GGCTGCTGCT
     TTAAGGCAGT TCGACGTCTC CACGGTACCG ACTCAGTGTG GACGACGTCA CCGACGACGA

 61  GCTGCTGCCC ACGCTCTGTG GCCCAGGCAC TGCTGCCTGG ACCACCTCAT CCTTGGCCTG
     CGACGACGGG TGCGAGACAC CGGGTCCGTG ACGACGGACC TGGTGGAGTA GGAACCGGAC

121  TGCCCAGGGC CCTGAGTTCT GGTGCCAAAG CCTGGAGCAA GCATTGCAGT GCAGAGCCCT
     ACGGGTCCCG GGACTCAAGA CCACGGTTTC GGACCTCGTT CGTAACGTCA CGTCTCGGGA

181  AGGGCATTTC CTACAGGAAG TCTGGGGACA TGTGGGAGCC GATGACCTAT GCCAAGAGTG
     TCCCGTAACG GATGTCCTTC AGACCCCTGT ACACCCTCGG CTACTGGATA CGGTTCTCAC

241  TGAGGACATC GTCCACATCC TTAACAAGAT GGCCAAGGAG GCCATTTTCC AGGACACGAT
     ACTCCTGTAG CAGGTGTAGG AATTGTTCTA CCGGTTCCTC CGGTAAAAGG TCCTGTGCTA

301  GAGGAAGTTC CTGGAGCAGG AGTGCAACGT CCTCCCCTTG AAGCTGCTCA TGCCCCAGTG
     CTCCTTCAAG GACCTCGTCC TCATGTTGCA GGAGGGGAAC TTCGACGAGT ACGGGGTCAC

361  CAACCAAGTG CTTGACGACT ACTTCCCCCT GGTCATCGAC TACTTCCAGA ACCAGACTGA
     GTTGGTTCAC GAACTGCTGA TGAAGGGGGA CCAGTAGCTG ATGAAGGTCT TGGTCTGACT

421  CTCAAACGGC ATCTGTATGC ACCTGGGCCT GTGCAAATCC CGGCAGCCAG AGCCAGAGCA
     GAGTTTGCCG TAGACATACG TGGACCCGGA CACGTTTAGG GCCGTCGGTC TCGGTCTCGT

481  GGAGCCAGGG ATGTCAGACC CCCTGCCCAA ACCTCTGCGG GACCCTCTGC CAGACCCTCT
     CCTCGGTCCC TACAGTCTGG GGGACGGGTT TGGAGACGCC CTGGGAGACG GTCTGGGAGA
```

MATCH WITH FIG. 5B

MATCH WITH FIG. 5A

FIG. 5B

```
541   GCTGGACAAG CTCGTCCTCC CTGTGCTGCC CGGGGCCCTC CAGGCGAGGC CTGGGCCTCA
      CGACCTGTTC GAGCAGGAGG GACACGACGG GCCCCGGGAG GTCCGCTCCG GACCCGGAGT

601   CACACAGGAT CTCTCCGAGC AGCAATTCCC CATTCCTCTC CCCTATTGCT GGCTCTGCAG
      GTGTGTCCTA GAGAGGCTCG TCGTTAAGGG GTAAGGAGAG GGGATAACGA CCGAGACGTC

661   GGCTCTGATC AAGCGGATCC AAGCCATGAT TCCCAAGGGT GCGCTACGTG TGGCAGTGGC
      CCGAGACTAG TTCGCCTAGG TTCGGTACTA AGGGTTCCCA CGCGATGCAC ACCGTCACCG

721   CCAGGTGTGC CGCGTGGTAC CTCTGGTGGC GGGCGGCATC TGCCAGTGCC TGGCTGAGCG
      GGTCCACACG GCGCACCATG GAGACCACTG CCCGCCGTAG ACGGTCACGG ACCGACTCGC

781   CTACTCCGTC ATCCTGCTCG ACACGCTGCT GGGCCGCATG CTGCCCCAGC TGGTCTGCCG
      GATGAGGCAG TAGGACGAGC TGTGCGACGA CCCGGCGTAC GACGGGGTCG ACCAGACGGC

841   CCTCGTCCTC CGGTGCTCCA TGGATGACAG CGCTGGCCCA AGGTCGCCGA CAGGAGAATG
      GGAGCAGGAG GCCACGAGGT ACCTACTGTC GCGACCGGGT TCCAGCGGCT GTCCTCTTAC

901   GCTGCCGCGA GACTCTGAGT GCCACCTCTG CATGTCCGTG ACCACCCAGG CCGGGAACAG
      CGACGGCGCT CTGAGACTCA CGGTGGAGAC GTACAGGCAC TGGTGGGTCC GGCCCTTGTC

961   CAGCGAGCAG GCCATACTAC AGGCAATGCT CCAGGCCTGT GTTGGCTCCT GGCTGGACAG
      GTCGCTCGTC CGGTATGATG TCCGTTACGA GGTCCGGACA ACCCCGAGGA CCGACCTGTC

1021  GGAAAAGTGC AAGCAATTTG TGGAGCAGCA CACGCCCCAG CTGCTGACCC TGGTGCCCAG
      CCTTTTCACG TTCGTTAAAC ACCTCGTCGT GTGCGGGGTC GACGACTGGG ACCACGGGTC
```

MATCH WITH FIG. 5C

FIG. 5C

MATCH WITH FIG. 5B

```
1081  GGGCTGGGAT GCCCACACCA CCTGCCAGGC CCTUGGGGT  TGTGGGACCA TGTCCAGCCC
      CCCGACCCTA CGGGT TGGT GGACGGTCCG GGAGCCCCA  ACACCCTGGT ACAGGTCGGG

1141  TCTCCAGTGT ATCCACAGCC CCGACCTTTG ATGAGAACTC AGCTGTCCAG GTGCAAAGGA
      AGAGGTCACA TAGGTGTCGG GGCTGGAAAC TACTCTTGAG TCGACAGGTC CACGTTTCCT

1201  AAAGCCAAGT GAGACGGGCT CTGGGACCAT GGTGACCAGG CTCTPCCCCT GCTCCCTGGC
      TTTCGGTTCA CTCTCCCCGA GACCCTGGTA CCACTGGTCC GAGAAGGGGA CGAGGGACCG

1261  CCTCGCCAGC TGCCAGGCTG AAAAGAAGCC TCAGCTCCCA GACCGCCCTC CTCACCGCCC
      GGAGCGGTCG ACGGTCCGAC TTTTCTTCGG AGTCGAGGGT GTGGCGGGAG GAGTGGCGGG

1321  TTCCTCGGCA GTCACTTCCA CTGGTGGACC ACGGGCCCCC AGCCCTGTGT CGGCCTTGTC
      AAGGAGCCGT CAGTGAAGGT GACCACCTGG TGCCCGGGGG TCGGGACACA GCCGGAACAG

1381  TGTCTCAGCT CAACCACAGT CTGACACCAG AGCCCACTTC CATCCTCTCT GGTGTGAGGC
      ACAGAGTCGA GTTGGTGTCA GACTGTGGTC TCGGGTGAAG GTAGGAGAGA CCACACTCCG

1441  ACAGCGAGGG CAGCATCTGG AGGAGCTCTG CAGCCTCCAC ACCTACCACG ACCTCCCAGG
      TGTCGCTCCC GTCGTAGACC TCCTCGAGAC GTCGGAGGTG TGGATGGTGC TGGAGGGTCC

1501  GCTGGGCTCA GGAAAAACAA GCCACTGCTT TACAGGACAG GGGGTTGAAG CTGAGCCCCG
      CGACCCGAGT CCTTTTTGGT CGGTGACGAA ATGTCCTGTC CCCCAACTTC GACTCGGGGC

1561  CCTCACACCC ACCCCCATGC ACTCAAAGAT TGGATTTTAC AGCTACTTGC AATTCAAAAT
      GGAGTGTGGG TGGGGGTACG TGAGTTTCTA ACCTAAAATG TCGATGAACG TTAAGTTTTA
```

MATCH WITH FIG. 5D

MATCH WITH FIG. 5C

```
1621  TCAGAAGAAT AAAAAATGGG AACATACAGA ACTCTAAAAG ATAGACATCA GAAATTGTTA
      AGTCTTCTTA TTTTTTACCC TTGTATGTCT TGAGATTTTC TATCTGTAGT CTTTAACAAT

1681  AGTTAAGCTT TTTCAAAAAA TCAGCAATTC CCAGCGTAGT CAAGGGTGGA CATGCACGCG
      TCAATTCGAA AAAGTTTTTT AGTCGTTAAG GGTCGCATCA GTTCCCACCT GTACGTGCGC

1741  TCTGGCATGA TGGGATGGCG ACCGGGCAAG CTTTCTTCCT CGAGATCGTC TGCTGCTTGA
      AGACCGTACT ACCCTACCGC TGGCCCGTTC GAAAGAAGGA GCTCTAGCAG ACGACGAACT

1801  GAGCTATTGC TTTGTTAAGA TATAAAAAGG GGTTTCTTTT TGTCTTTCTG TAAGGTGGAC
      CTCGATAACG AAACAATTCT ATATTTTTCC CCAAAGAAAA ACAGAAAGAC ATTCCACCTG

1861  TTCCAGCTTT TGATTGAAAG TCCTAGGGTG ATTCTATTTC TGCTGTGATT TATCTGCTGA
      AAGGTCGAAA ACTAACTTTC AGGATCCCAC TAAGATAAAG ACGACACTAA ATAGACGACT

1921  AAGTCTAGCT GGGGTTGTGC AAGCTAGGGA CCCATTCCTA TGTAATACAA TGTCTGCACC
      TTCGAGTCGA CCCCAACACG TTCGATCCCT GGGTAAGGAT ACATTATGTT ACAGACGTGG

1981  AATGCTAATA AAGTCCTATT CTCTTTTATC GGAATT
      TTACGATTAT TTCAGGATAA GAGAAAATAG CCTTAA
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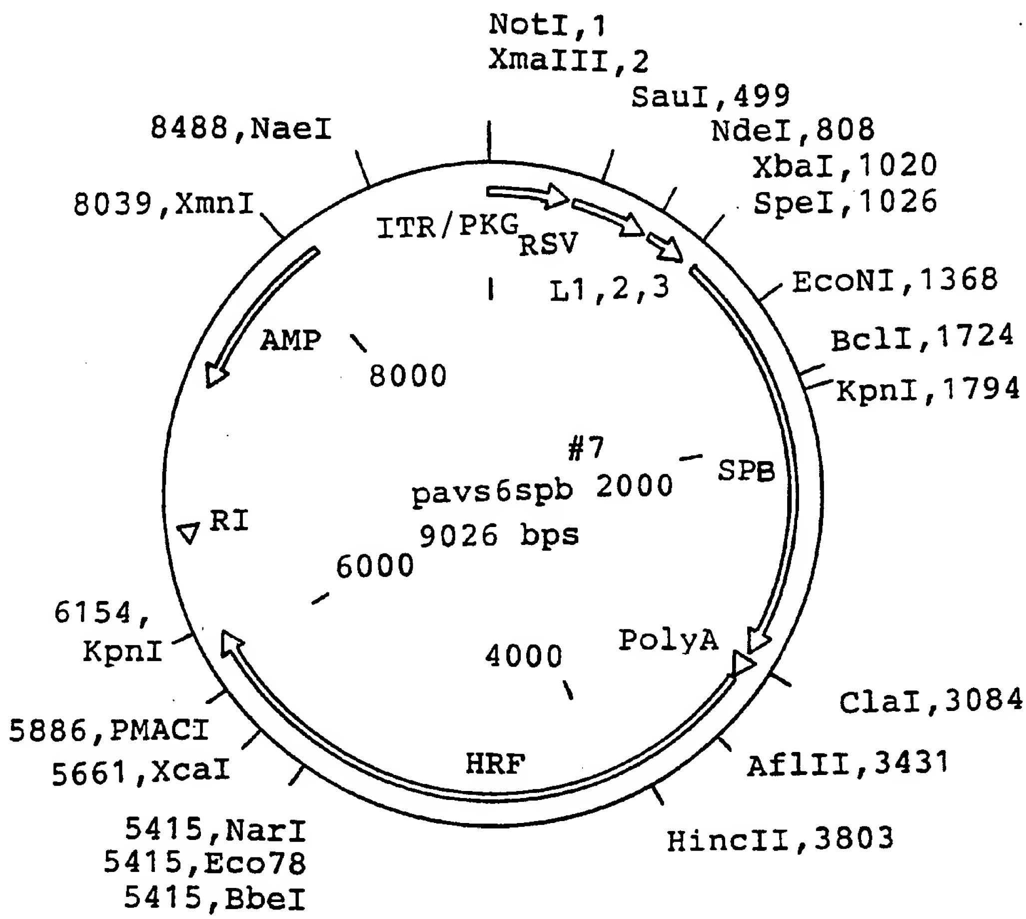
FIG. 5D

FIG. 7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SpeI,1026
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ClaI,3084
AflII,3431
HincII,3803
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6154,
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ITR/PKG
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RI
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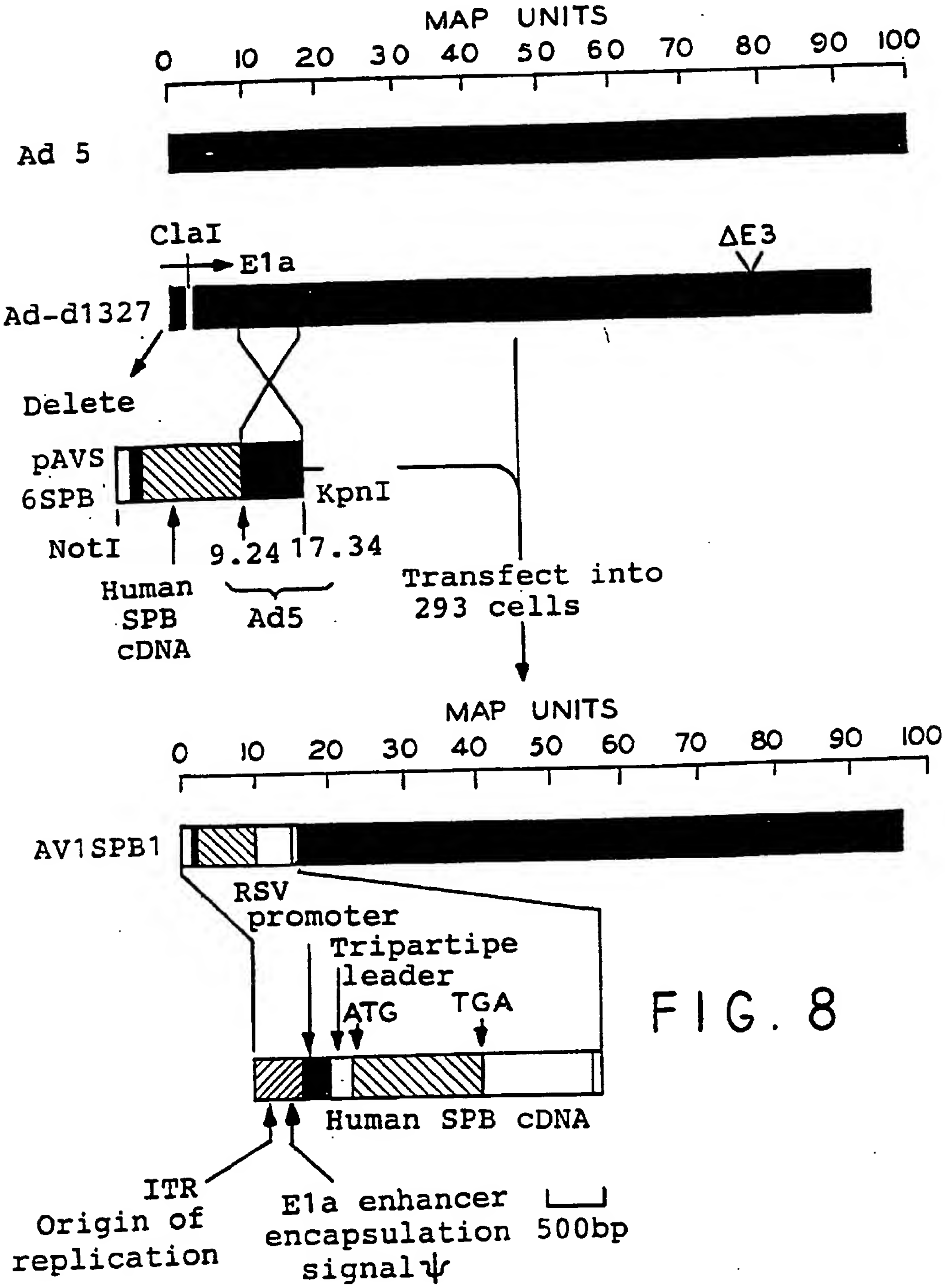
HRF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Ad 5
ClaI
E1a
ΔE3
Ad-d1327
Delete
pAVS
6SPB
KpnI
NotI
9.24
17.34
Human
SPB
cDNA
Ad5
Transfect into
293 cells
MAP UNITS
0 10 20 30 40 50 60 70 80 90 100
AV1SPB1
RSV
promoter
Tripartipe
leader
ATG
TGA
Human SPB cDNA
ITR
Origin of
replication
E1a enhancer
encapsulation
signal ψ
500bp

FIG. 8

FIG. 9

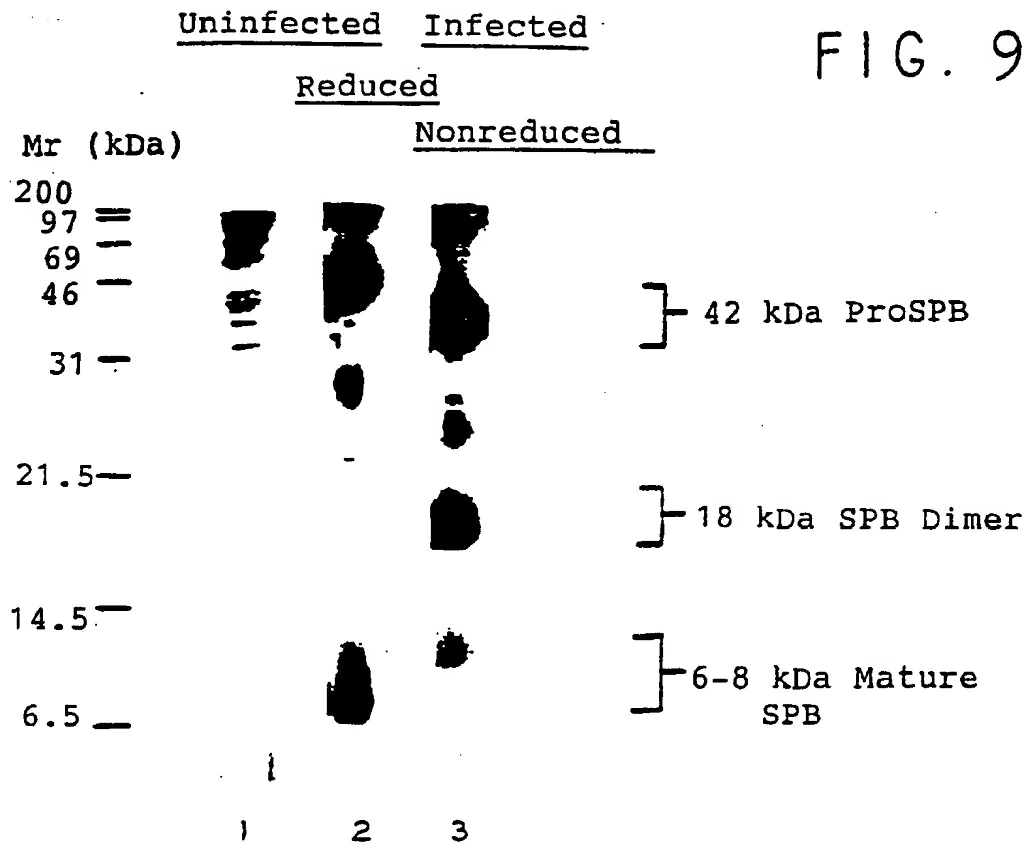
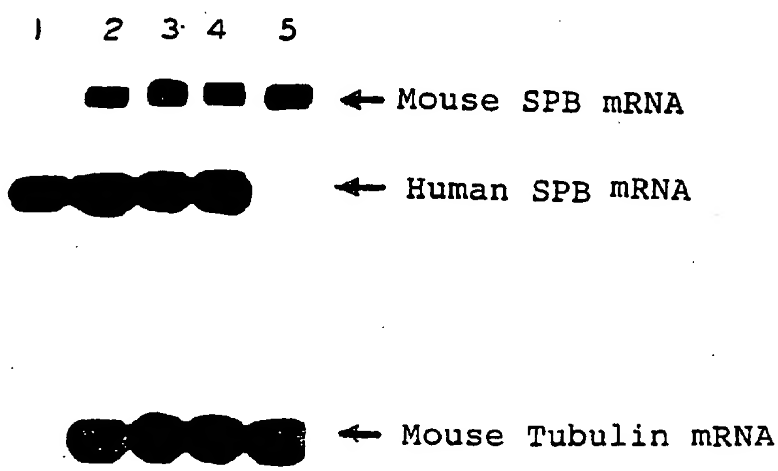
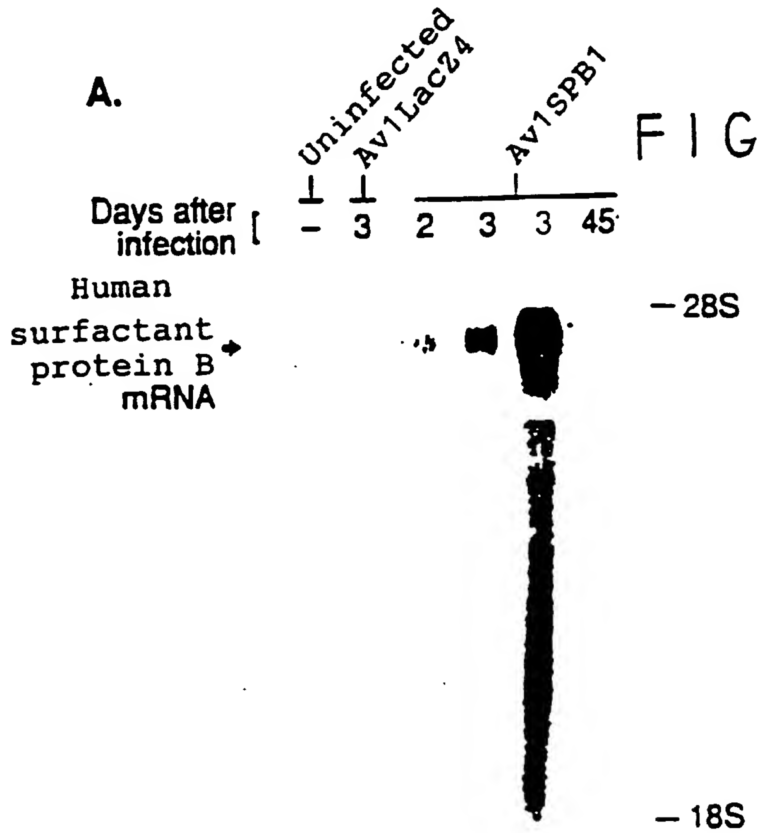
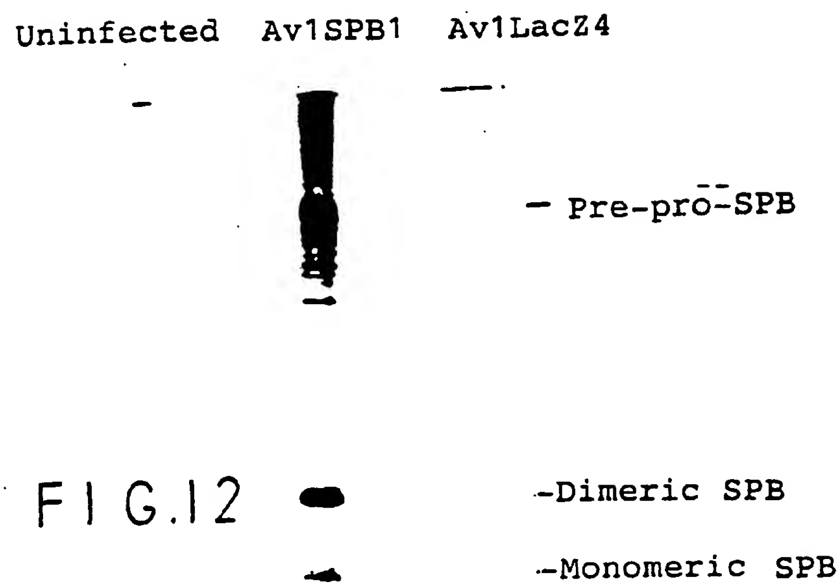

Uninfected
Infected
Reduced
Nonreduced
Mr (kDa)
200
97
69
46
31
21.5
14.5
6.5
42 kDa ProSPB
18 kDa SPB Dimer
6-8 kDa Mature SPB
1
2
3

FIG. 10


1
2
3
4
5
Mouse SPB mRNA
Human SPB mRNA
Mouse Tubulin mRNA


A.
Uninfected
Av1LacZ4
Av1SPB1
Days after infection
– 3 2 3 3 45
Human surfactant protein B mRNA
– 28S
– 18S

F I G. 11


Uninfected
Av1SPB1
Av1LacZ4
– Pre-pro-SPB
–Dimeric SPB
–Monomeric SPB

F I G.12

FIG. 13

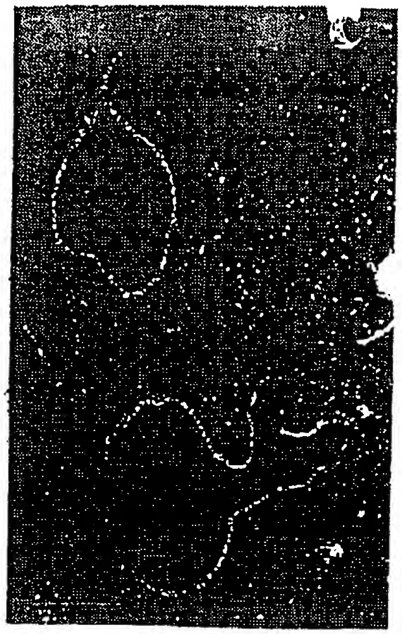


FIG. 14

FIG. 15

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03831

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 63/00; A61K 37/00; C07H 17/00; C12N 5/00, 7/00, 15/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93A, 93R; 435/172.1, 172.3, 235.1, 240.2, 320.1; 536/23.1, 23.5, 24.1, 24.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	W. Doerfler, "Adenovirus DNA", published 1986, by Martinus Nijhoff Publishing (Boston), pages 53-95, see the entire document.	1-24
Y	Cell, Volume 68, issued 10 January 1992, M.A. Rosenfeld et al, "In Vivo Transfer of the Human Cystic Fibrosis Transmembrane Conductance Regulator Gene to the Airway Epithelium", pages 143-155, see the entire document.	1-24
Y	Science, Volume 252, issued 19 April 1991, M. A. Rosenfeld et al, "Adenovirus-Mediated Transfer of a Recombinant α1-Antitrypsin Gene to the Lung Epithelium in Vivo", pages 431-434, see the entire document.	1-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

25 MAY 1994

Date of mailing of the international search report

JUN 22 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
BRUCE CAMPELL D. Kyza for
Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DNA, Volume 8, No. 2, issued 1989, T.J. Pilot-Matias et al, "Structure and Organization of the Gene Encoding Human Pulmonary Surfactant Proteolipid SP-B", pages 75-86, see the entire document.	1-24
Y	Journal of Clinical Investigation, Volume 81, issued March 1988, S.D. Revak et al, "Use of Human Surfactant Low Molecular Weight Apoproteins in the Reconstitution of Surfactant Biologic Activity", pages 826-833, see the entire document.	15-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03831

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/93A, 93R; 435/172.1, 172.3, 235.1, 240.2, 320.1; 536/23.1, 23.5, 24.1, 24.2

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, EXCERPTA MEDICA, DERWENT BIOTECHNOLOGY ABSTRACTS, CHEMICAL ABSTRACTS
search terms: surfactant and (pulmonary or lung), adenovirus vector?, gene therapy